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Original Article

The effects of cigarette smoke on the epididymal tissues in adult male albino rats and the ameliorative effect of the Sidr honey.

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Abstract

The present study investigated the effects of cigarette smoke (CS) on the epididymal tissues and the protective effect of Sidr honey in adult male rats. 24 adult male rats were divided into four groups: Group 1: normal control group; Group 2:rats received Sidr honey orally (100 mg/kg b.w./d.) for 4 weeks; Group 3: rats were exposed to five lit cigarettes per day (5 times per day) for 4 weeks; and Group 4: rats received Sidr honey orally (100 mg/kg b.w./d.) for 2 weeks, then the rats were treated with cigarette smoke generated by a machine after taking the Sidr honey for 4 weeks. The results presented the gross morphology of the vas L deferens of rats in the group 3was smaller than that of the other groups. Moreover, the testes in group 3 were bigger as compared to all other groups. In addition, results demonstrate the rats in group 3 showed many histopathological changes in epididymal tissues when compared with the group 1. While the epididymal tissues from the rats in the group 4displayednearly normal histological structure of the tubules with normal sperm density as compared to the group 3. This study indicates that Sidr honey has a protective effect against CS-induced epididymal damage in adult male albino rats.

Key Words: Cigarette smoke, Epididymal tissues, Sidr honey, Adult rats.

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Introduction

Cigarette smoke (CS) is today by far the most popular form of smoking among many young adults; however, it is the greatest threat to public health [1] and it constitutes above 80% of passive smoke [2]. Moreover, the reproductive

dysfunction is a major cause of infertility among couples and cigarette smoke has been shown to cause different forms of reproductive dysfunction in both males and females [3,4]. Besides,the male reproductive system is highly sensitive to chemicals and drugs that have been defined to cause adverse effects on male reproductive capacity [5]. Aside from the principal biologically active component (nicotine), tobacco products also contain several potentially toxic compounds, including polycyclic aromatic hydrocarbons, cyanide, carbon monoxide, heavy metals, nitrosamines, and insecticides [6]. In addition, the toxicity of cigarette smoke products is due to the huge production of reactive oxygen species (ROS) in humans [7] and may produce inflammatory mediators [8]. Exposure to cigarette smoke causes the release of many harmful substances in the body that have the direct potential of forming free radicals and activating inflammatory cells, which produce ROS [9]. Therefore, ROS reduces male sex hormone levels and disrupts the hormonal balance for male reproductive functions,thus causing infertility [10]. Furthermore, oxidative stress, arising from the lopsided balance of endogenous antioxidant systems and reactive oxygen species, had long been associated with male infertility, where, the spermatozoa have a fragile antioxidant defense system and are susceptible to oxidative stress and subsequent compromise of sperm integrity and epigenome[11]. However, little is known about the effect of CS on male reproductive organs such as the prostate, epididymis,and seminal vesicle [12]. Honey is a natural product that is widely used for its therapeutic effects [13]. Ithas been usedas food, drug, and raw materials [14]. As a medicinal material, its antioxidant ability and supersaturated sugar solution with high osmotic pressure build up the immunity level of consumers [15, 16]. It has been reported that honey contains about 200 compounds [13]. Additionally, phenolic compounds are the main antioxidant compounds in honey as extremely important correlations are found between total phenolic contents and antioxidant and antiradical activities of honey [12]. Moreover, the curative potential of honey involves free radical scavenging activities and antibacterial properties, as well as wound-healing and anti-inflammatory activities [17, 18]. According to [13] the honey may be effective against a wide range of diseases, from a wound to cancer.

There are no scientific reports on the effectiveness of Libyan sidr honey to validate its traditional use in the cure and prevention of reproductive tissue damage. Therefore,the present study examined the protective effects ofLibyan Sidr honeyagainst epididymal tissues damage caused by exposure to cigarette smoke in male rats.

Materials and Methods

Chemicals:

The Libyan Sidr honey used was obtained from the local agricultural market and was analyzed by the Centre Lab of Omar Al-Mokhtar University, El-Beyda, Libya. 100mg/kg of honey was administered

to the rats[19]. This dose was worked out relative to the local human consumption of honey. Honey at the dose of 1.0 g/kg body weight was freshly diluted with distilled water to prepare 0.5 mL of diluted honey for each rat. Then, 0.5 mL of the diluted honey was immediately administered to each rat by oral gavages.

Karelia red cigarettes were obtained from the local supermarkets.

Experimental animals:

24 adult male albino rats (Rattus norvegicus),10 weeks old weighing 180-200g were used. Rats were obtained from the animal house of the Zoology Department, Faculty Science, University of Omar Al-Mokhtar, El-Beyda, Libya. They were acclimatized for a period of 3 weeks and were housed in cages at standard laboratory conditions of room temperature (22 ±2oC). Animals were fed standard rat chow and water ad libitum. The procedures and animal protocols were followed in this study in accordance with the guide for the care and use of laboratory animals.

Experimental design:

Rats were randomly assigned into four groups of 6 animals as follows:

Group 1: The normal control group (NC), rats were kept under standard laboratory conditions with ventilation and were not exposed to smoke.

Group 2: The Sidr honey group (H), rats were given Sidr honey (100mg/kg b.w./d.) [19] orally by gavages for 4 weeks.

Group 3: The Cigarette smoke group (CS). Cigarette smoke exposure was conducted by cigarette smoke generated by a machine (bee smoker) device and a hole was connected to a smoking machine by the connection pipe to the glass box which was designed locally in the Zoology Department, Faculty Science, University of Omar Al-Mokhtar, El-Beyda, Libya (Figures 1& 2). The inhalation was performed in the closed glass box for condensation of the smoke a cover was removed to provide an unforced exchange of fresh air. The glass box is in a cube shape (aquarium shape) with the size of 80 × 30 × 40 cm for keeping the rats [20].

The cigarette smoke was used five lit Karelia red cigarettes (3.8 mg). Each smoking procedure was for 15 minutes including making the smoke and exposing the rats to the smoke for 5 minutes and then, 10 minutes of rest and ventilation by removing the box cover. This operation was repeated 5 times a day for 4 weeks. Each week, for 6 days the rats were exposed to the smoke [21].

Group 4: The protective group (P), rats were given Sidr honey (100mg/kg b.w./d.) orally by gavages for 2 weeks than animals treated with cigarette smoke generated by a machine smoking (same group 3) after taking the Sidr honey for 4 weeks.

Figure 1: The glass box and smoking machine (bee smoker).

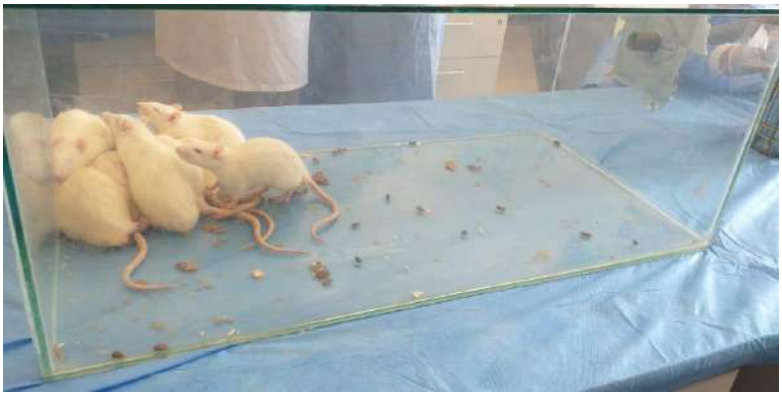

Figure 2**:** Rats were exposure to cigarette smoke.

Evaluation of the reproductive organs morphology:

After the completion of the treatment period, all rats were anesthetized with diethyl ether, then sacrificed, and their reproductive organs, including the testes, epididymis, and vas deferens, were removed. were grossly observed for their sizes and morphologies. All organs of all groups were captured by camera to be compared for morphological changes.

Histopathological examination

Epididymis specimens from all groups were fixed in formalin (10%), then the cauda epididymis was dehydrated in graded alcohol and embedded in paraffin. Sections of 5 nm thickness were stained with hematoxylin and eosin using standard procedures. The sections were examined under a light microscope [22]. Changes in the experimental histopathologic parameters for kidney tissues were graded as follows: (–) indicates normal, (+) indicates mild, (++) indicates moderate, and (+++) indicates severe changes. [23, 24].

Result

Evaluation of the reproductive organs morphology:

The comparisons of gross morphological aspects of the testis,epididymis, vas deferens, and seminal vesicle among the control and experimental groups were demonstrated in figure 3.. The results showed that testis,epididymis, vas deferens, and seminal vesicles in groups 2 and 4 were not obviously different from each other as compared to those organs of the group 1 (Figure 3 A, B, and D). In contrast, the gross morphology of the vas L deferens of rats in group 3 was obviously smaller than that of rats in groups 1, 2, and 4. Moreover, the testes in group 3 were obviously bigger as compared to all other groups shown in Figure 3 C.

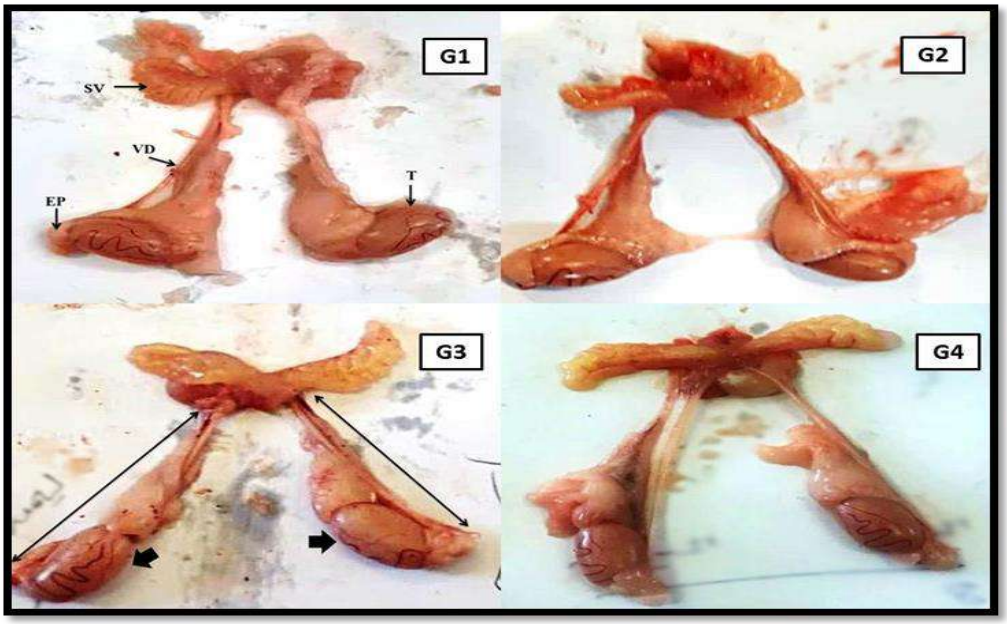

Figure 3: Gross morphology of the reproductive organs in adult male rats, including the seminal vesicle (SV), vas deferens (VD), testis (T), and epididymis (EP), shows that the vas L deferens (↕) of rats in group 3 was obviously smaller than that of other groups. While the testes (arrows) in group 3 were obviously bigger as compared to all other groups,

* (G1- Normal control group, G2- Sidr honey group, G3- cigarette smoke group, and G4- protective group).

Histopathological examination:

Microscopically, the epididymis sections showed normal histological structure with normal sperms density, normal ducts with normal epithelial height lined with pseudostratified columnar epithelium, normal epithelial cells and nuclear size, and stroma with normal aspect in the group 1(Figures 4 and 5). Additionally, in all rats in group 2, there were no evident microscopical changes in epididymal

tissues as compared to rats in group 1 (Figures 6 and 7).inflammatory cell infiltration in stroma, emptying epidydmal ducts and contained few sloughed germ cells in the lumen, degeneration of epithelial cells, cellular debris and distorted basement membrane, and hyperplasia were appeared (Figure 9), on the other hand, vacuolation of lining germinal epithelial cells as well as single cell necrosis (Figure 10). However, dilated and congestion blood vessel as seen in figure (Figure 11). Also, the figure (12) showed reduction of sperms mass and vacuoles of cytoplasm with faintly stained. Furthermore, shattered of the epithelium layer, and degeneration and disorganization of the epithelium layer were noticed in figure (13). Thus, the results indicate that they illustrate the different histopathological changes in the epididymal tissue of animals in Group 3. Whereas, the epididymal tissue of rats in group 4 showed a nearly normal histological structure with normal sperm density, normal epididymal tubules lined by low pseudostratified columnar epithelium, and stroma with some neutrophilic and lymphocytic inflammatory cell infiltration (Figures 14 and 15). The epididymis sections of group 4 showed moderate improvement in their tissue structure.

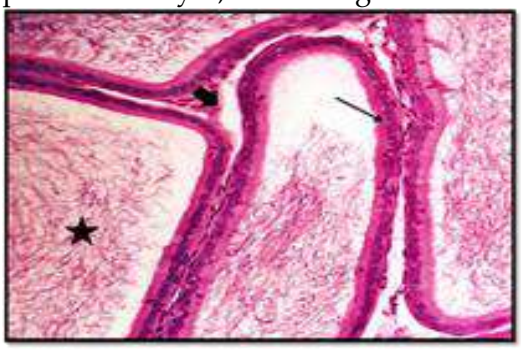

Figure 4: Photomicrograph of the epididymis section of group 1 showing, normal histological structure with normal sperm density (star), normal epithelial cells (arrow), and stroma with normal aspect (thick arrow). (H & E, X400).

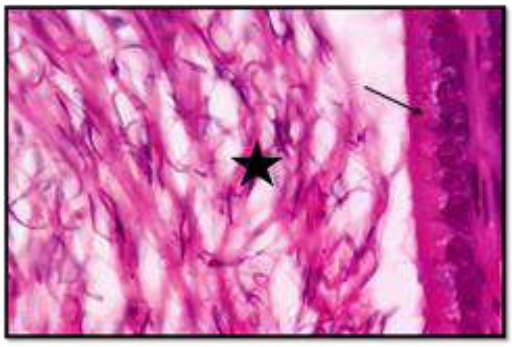

Figure 5**:** Photomicrograph of the epididymis section of group 1 showing, normal histological structure with normal sperm density (star), and normal epithelial cells (arrow). (H & E, X 1000).

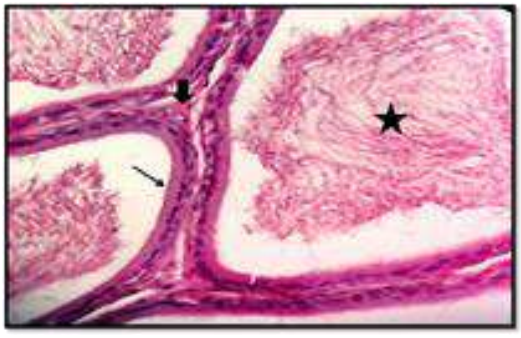

Figure 6: Photomicrograph of the epididymis section of group 2 showing, normal histological structure with normal sperm density (star), normal

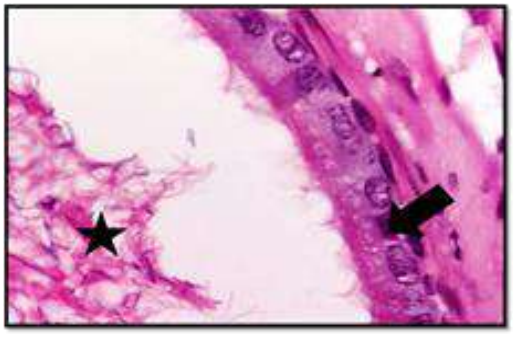

Figure 7: Photomicrograph of the epididymis section of group 2 showing, normal histological structure with normal sperm density (star),

epithelial cells (arrow), and stroma with normal aspect (thick arrow). (H & E, X400).

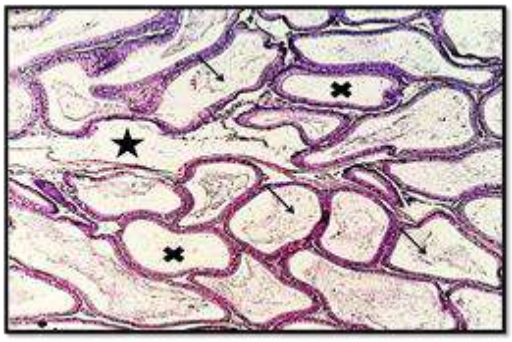

Figure 8: Photomicrograph of the epididymis section of group 3 showing, oedema and widely separating epididymal ducts (star), and some emptying epidydmal ducts (X). While, reduced sperms density in other epididymal ducts (arrows).(H & E, X100).

normal epithelial cells (arrow), and stroma with normal aspect (thick arrow). (H & E, X1000).

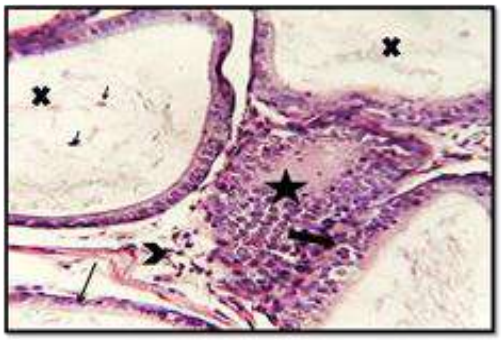

Figure 9: Photomicrograph of the epididymis section of group 3 showing oedema and widely separating epididymal ducts (head arrow) with inflammatory cell infiltration, emptying epididymal ducts (X), and containing few sloughed germ cells in the lumen (short arrows), degeneration of epithelial cells (long arrow), cellular debris and distorted basement membrane (thick arrow), and hyperplasia (star) (arrow). (H & E, X400).

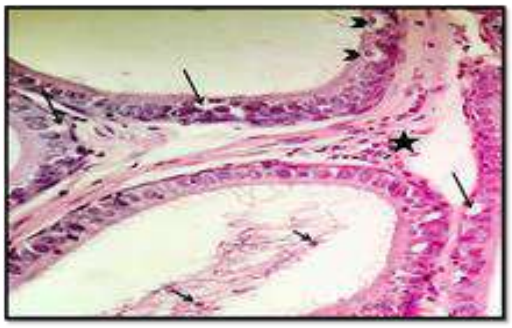

Figure 10: Photomicrograph of the epididymis section of group 3 showing, vacuolation of lining germinal epithelial cells (long arrows) as well as single cell necrosis (head arrows), oedema with inflammatory cell infiltration (star), reduced sperms, and a few sloughed germ cells in the lumen (short arrows). (H & E, X400).

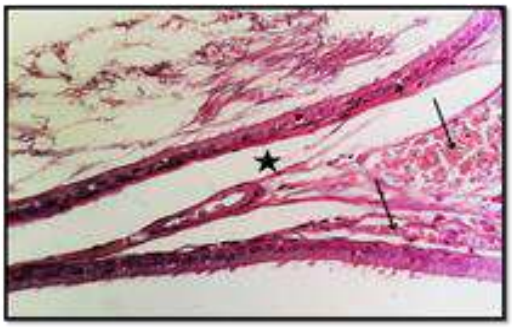

Figure 11: Photomicrograph of the epididymis section of group 3 showing, dilated and congested blood vessels (long arrows), vacuolation of few lining germinal epithelial cells (short arrows), and oedema and widely separating epididymal ducts (star). (H & E, X400).

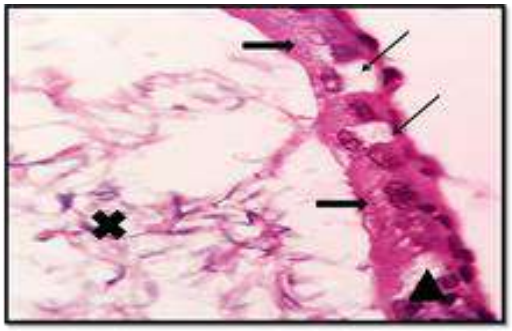

Figure 12: Photomicrograph of the epididymis section of group 3 showing, vacuolation of few lining germinal epithelial cells (head arrow), single cell necrosis (long arrows), reduction of sperm mass (star), and vacuoles of cytoplasm with faintly stained (H & E, X1000).

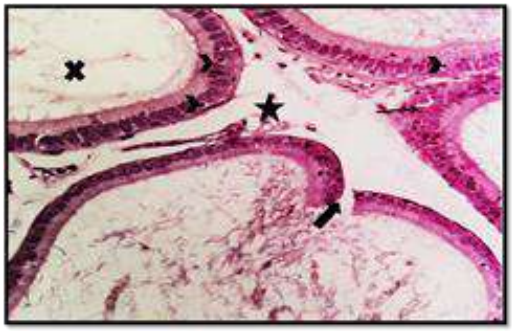

Figure 13: Photomicrograph of the epididymis section of group 3 showing, pyknotic nuclei in some epithelial cells (head arrows), and oedema and widely separating epididymal ducts (star), shattered epithelium layer (thick arrow), decreased sperms in the lumen (X), and degeneration and disorganization of the epithelium layer (long arrow). (H & E, X400).

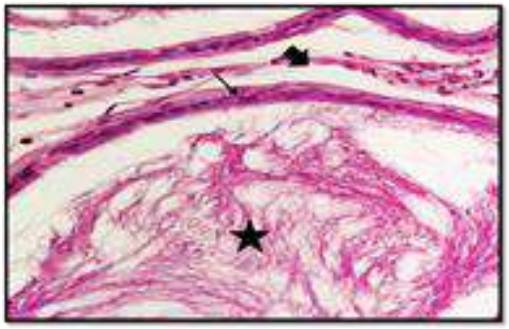

Figure 14: Photomicrograph of the epididymis section of group 4 showing, nearly normal histological structure with normal sperm density (star), and normal epididymal ducts lined by low pseudostratified columnar epithelium (thick arrow), stroma with some inflammatory cell infiltration (thick arrow). (H & E, X400).

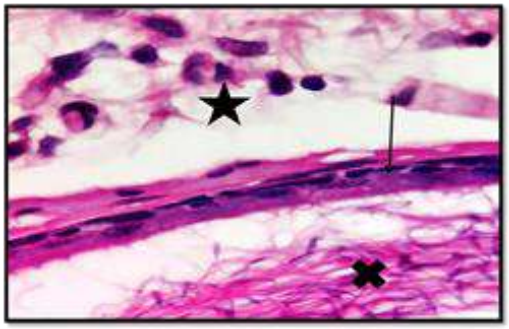

Figure 15: Photomicrograph of the epididymis section of group 4 showing, nearly normal histological structure with normal sperm density (X), and normal epididymal ducts lined by low pseudostratified columnar epithelium (thick arrow), and stroma with neutrophilic and lymphocytic inflammatory cell infiltration (star). (H & E, X1000).

Histopathological lesions in the epididymis:

The changes in the histological structures of the epididymal tissues were graded in (Table. 1). The epididymis sections of rats in the group 4 showed a great but moderate improvement in their tissues structure when compared with group 3.

Table 1: Incidence of histopathological lesions in the epididymis of the control and experimental groups.

Organs	Lesions	G1	G2	G3	G4
Epididymis	Degeneration of epithelial cells	-	-	+++	-
	Oedema	-	-	++	-
	Absence of the spermatozoa	-	-	++	-
	Reduction of spermatozoa	-	-	++	-
	Necrosis	-	-	++	-
	Congestion	-	-	+	-
	Inflammatory cells infiltration	-	-	+++	++
	Vacuolations	-	-	++	-

*(–) indicates normal, (+) indicates mild, (++) indicates moderate, (+++) indicates severechanges.
* (G1- Normal control group, G2- Sidr honey group, G3- cigarette smoke group, and G4- protective group).

Discussion

The consumption of foods and substances rich in antioxidants may protect against pathological changes and thus prevent the pathogenesis of chronic inflammatory diseases. Some researchers have reported that honey contains several important substances, including antioxidants and other phytochemicals [17]. Our results showed that testis,epididymis, vas deferens, and seminal vesicle in the group 2 and group 4 were not obviously seen to be different from each other as compared to those organs of group 1. In contrast, the gross morphology of the vas deferens of rats in group 3 was obviously smaller than that of the other groups. Moreover, the testes in group 3 were obviously bigger as compared to all the other groups. These findings have also been experimental when the rats were treated with other chemicals [25], who suggested that the long-term deficiency induced obstruction in the vasdeferens duo to structural defects in the epithelial of these tissues. In addition, this study demonstrates the rats in the group histopathological changes in epididymal tissues when compared with group 1, such as an oedema with widely separating epididymal ducts, some emptying epidydmal ducts, reduced sperm density in other epidydmal ducts, inflammatory cell infiltration in the stroma, degeneration of epithelial cells, cellular debris, a distorted basement membrane, and hyperplasia, were evident, as were vacuolation of the lining of germinal epithelial cells as well as single-cell necrosis, dilated, and congestion of the These histological changes have also been observed when the rats were treated with other toxic substances, suggesting the presence of increased phagocytosis within the epididymal epithelial cells, where some of the abnormal sperms are found to be phagocytosed by the epididymal epithelial cells [12]. Moreover, [26] suggested that the low number of spermatozoa in most of the epididymal ducts and degenerating detached germ cells in the lumina of the epididymal tubule signifies testicular dysfunction, which might be mediated through scavenging generated ROS and protecting against inflammatory reactions. The rounded cells in the lumen of the epididymal duct of

animals exposed to CS could be cells that have broken off from the epididymal epithelium or immature germ cells originating from the testicle [27].

Shashi and Khan [28] reported that it was documented that the infiltration of inflammatory cells in the epididymis causes the apoptosis and necrosis of germinal cells. Also, they said the reduction in sperm density and cell debris in the lumen as compared to controls could have that effect on the structural changes in the epithelium and then its internal milieu, thereby making it nonconducive for physiological sperm maturation and survival. On the other hand, cigarette smoke results in an impaired epididymal sperm maturation process and may be associated with a diminished capacity of the spermatozoa to penetrate oocytes [29]. According to [30],CS is a major exogenous source of reactive oxygen species, which are capable of inducing lipid peroxidation, DNA damage, apoptosis of sperm cells and increased oxidative stress and DNA damage. Also, [29] said the decrease in the population of mature sperm cells could be due to a reduced population of early spermatogenic cells series, disruption, or destruction or death of growing cells, or some other developmental abnormalities occasioned by oxidative stress, tissue ischemia, apoptosis, or any of the proposed mechanisms.

On the other hand, the epididymal tissues of rats in Group 4 showed a nearly normal histological structure with normal sperm density, normal epididymal tubules lined by low pseudostratified columnar epithelium, and a stroma with some neutrophilic and lymphocytic inflammatory cell infiltration. The epididymis sections of the rats in Group 4 showed moderate improvement in their tissue structure. These results are in agreement with [12],who reported the administration of honey before CS showed a marked reduction in the histopathological changes in the epididymis tissue. These results might suggest that honey might have protective effects on the oxidative stress in rat tissues exposed to CS; they said the honey has some vitamins and antioxidants such as vitamins A, C, and E, flavonoids, and phenols [20, 19]. Also, they suggested that honey has a protective effect against CS-induced toxicity on the epididymis in rats. Moreover,the anti-inflammatory and antioxidant properties of honey are due to its phenolic compounds, flavonoids,carotenoid derivatives and organic acids[18]. On the other hand, [17] reported that honey reduced the activities of cyclooxygenase-1 and cyclooxygenase-2, thus showing anti-inflammatory effects. Additionally, [30] suggests the pre-treatment with Sidr Honey showed an antiapoptotic effect against ethanol-induced cell injury by acting as antisecretory,cytoprotective, and antioxidant agents. These might suggest that the Sidr honey has the potential healing properties against the toxic effects of CS to reduce epididymis injury.

Conclusion

The present findings clearly determine that exposure to cigarette smoke acts as a potential reproductive toxicant, leading to significant alterations in the internal milieu of epididymal tissues and marked changes in the maturation process of spermatozoa in rats. Moreover, that post treatment with the Sidr honey has a protective effect against CS-induced epididymisinjury in adult male albino rats. So, we should pay attention to the protective effects of natural antioxidants to support the endogenous antioxidant, assist in the perfection of organ functions, and avoid apoptosis.

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